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CASE REPORTS

Graphium basitruncatum Fungemia in a Patient with Acute Leukemia▽

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We report the first case of infection caused by *Graphium basitruncatum* in a man with acute leukemia who developed persistent fungemia and skin lesions. *G. basitruncatum*, a member of the *Microascaceae*, is phylogenetically and morphologically distinct from *Graphium penicillioides* and the opportunistic pathogens *Scedosporium apiospermum* (*Pseudallescheria boydii*) and *Scedosporium prolificans*.

CASE REPORT

A 70-year-old man, previously in good health, was diagnosed with acute myelogenous leukemia, FAB M0, in November 2005. The patient was originally from Germany and had immigrated to Canada in 1983. He was admitted to the hospital and received induction chemotherapy with daunorubicin from days 1 to 3 and cytarabine from days 1 to 7. He was also given itraconazole, 200-mg oral solution, twice daily as antifungal prophylaxis for 4 weeks starting on day 4 of chemotherapy. The postinduction course was complicated by the development of fever and neutropenia (absolute neutrophil count, <500 cells/μl), for which he received piperacillin-tazobactam and ciprofloxacin intravenously. This resulted in resolution of fever after 1 week. Investigations, including blood cultures, urine cultures, and computed tomography of the chest, did not reveal a source of infection. He failed to have adequate neutrophil recovery after the first course of induction chemotherapy and was discharged from the hospital on no antimicrobials, with an absolute neutrophil count of 0 cells/μl.

Three weeks later, the patient was rehospitalized with febrile neutropenia. His physical examination was remarkable for several nontender, erythematous skin nodules, primarily along his extremities (Fig. 1). Blood cultures were done in broth bottles and kept in a continuous monitoring system (BacT/ALERT; BioMérieux Inc., Durham, NC). He was started on broad-spectrum antimicrobial therapy consisting of piperacillin-tazobactam, ciprofloxacin, and vancomycin. Two blood cultures from the central venous catheter taken 4 days apart were positive and showed the presence of fungal hyphae. He was then started on a combination of intravenous voriconazole, 4 mg/kg of body weight twice daily, and caspofungin, 50 mg daily. He continued to be febrile but subsequently had three negative blood cultures. Computed tomography of his chest was normal. After 10 days of antifungal therapy, he received repeat induction chemotherapy with mitoxantrone and etoposide on days 1 to 5, followed by high-dose cytarabine on days 6 and 7. Despite ongoing combination antifungal therapy with voriconazole and caspofungin, the patient's fungemia recurred, and seven subsequent blood cultures over a 1-month period remained positive for a grayish fungus that was later identified as *Graphium basitruncatum*. The patient's central venous catheter was removed. A transesophageal echocardiogram showed no vegetations. Intravenous liposomal amphotericin B, 5 mg/kg daily, was started, and this resulted in defervesence, regression of skin lesions, and no evidence of ongoing fungemia. The patient subsequently recovered his neutrophil count (absolute neutrophil count, 5,900 cells/μl) and was discharged in February 2006 on daily injections of liposomal amphotericin. In March 2006, 6 weeks after recovery of peripheral blood cell counts, the patient redeveloped subcutaneous nodules and a lesion above his left lateral malleolus that drained necrotic, purulent material. A swab of this lesion showed the presence of fungal elements by calcofluor stain but was culture negative. Blood cultures remained negative. A bone marrow biopsy indicated that the leukemia was in remission. His condition progressively deteriorated, with increasing sizes of cutaneous lesions, despite ongoing antifungal therapy with liposomal amphotericin B. He opted for palliative management. Antifungal therapy was discontinued, and the patient subsequently died. No autopsy was performed.

Mycology. Isolates from three blood cultures were referred to the University of Alberta Microfungus Collection and Herbarium, and two were given accession numbers UAMH 10611 and UAMH 10620. When grown on potato dextrose agar (Difco Laboratories, Detroit, MI) at 30°C, colonies were 2.5 cm in diameter after 7 days and pale grayish brown with low-floccose mycelium, reaching diameters of 7 cm after 21 days and becoming dark grayish brown on the obverse and reverse. Growth was slower at 35°C, with colonies attaining diameters of 4 cm after 21 days. There was no growth on medium containing 0.4% cycloheximide. Microscopic mounts were ob-

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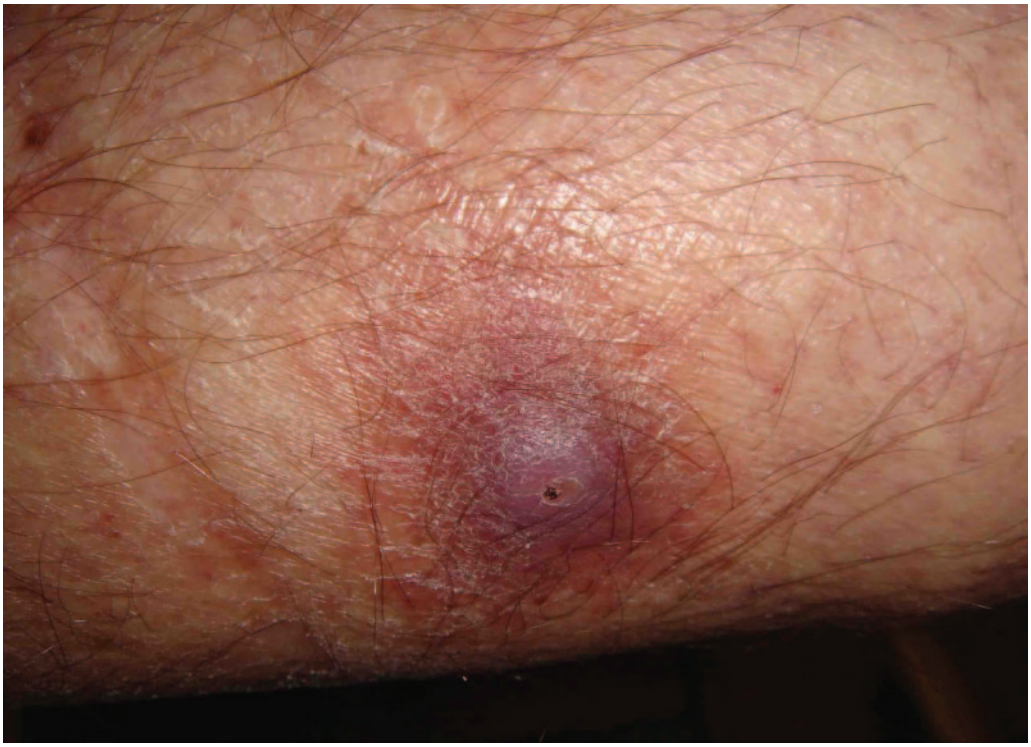

FIG. 1. Nodular, erythematous skin lesion of the left arm.

tained from cereal agar in slide culture preparations and from oatmeal salts agar (recipes are given in reference 7) after 7 to 21 days of incubation. Examination revealed conidia of two types produced on mononematous and synnematous conidiophores (Fig. 2). Conidiogenous cells were percurrently proliferating (annellides), measuring 8 to 20 μm long by 1 to 2 μm wide. These were produced singly or in whorls of two to several cells on mononematous conidiophores or at the apex of the synnemata. The predominant conidia were hyaline, single-celled, allantoid (sausage shaped), with truncate bases, and measured 4 to 7.5 μm long by 1.3 to 2.2 μm wide (average, 5.5 by 1.9 μm). Oval to ellipsoidal brown conidia were produced from the same or different conidiophores and measured 4.5 to 6.5 μm long by 2.5 to 4 μm wide (average, 5.4 by 3.3 μm).

The isolates were identified as *Graphium basitruncatum* by morphological comparison with the ex-type culture, obtained from the Japan Collection of Microorganisms as JCM 9300 (= UAMH 8494), and by sequencing of the internal transcribed spacer regions of the nuclear rRNA gene. The morphological features of the ex-type culture matched those of the patient isolates. Average conidial dimensions were 5.2 μm long and 1.7 μm wide for the curved conidia and 5.6 μm long and 3.2 μm wide for the brown conidia. The ex-type culture failed to grow on medium with 0.4% cycloheximide but grew slightly

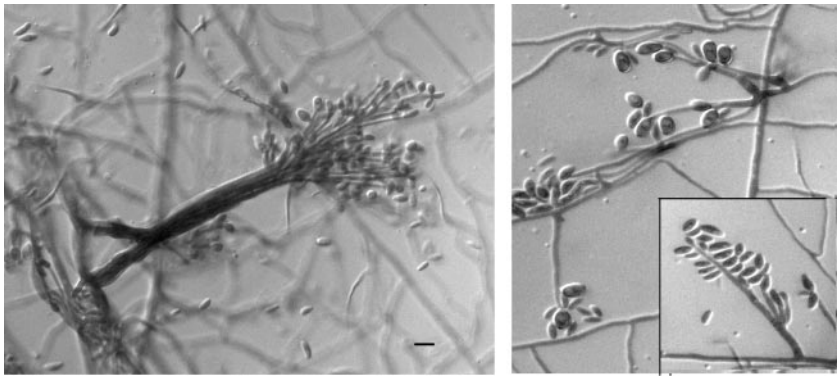

FIG. 2. Synnematous (left) and mononematous (right) conidiophores of *Graphium basitruncatum*. Microscopic preparations are from oatmeal salts agar (left) and slide culture mounts (right) after 1 week. Note the presence of conidia of two types, including hyaline sausage-shaped conidia with truncate bases and brown oval conidia. Bar = 5 μm.

faster than the patient isolates on potato dextrose agar (3-cm diameter after 7 days at 30°C). Procedures for DNA extraction, amplification, and sequencing were according to the protocols outlined by Sigler and Gibas (14). A BLAST search (1) with the 580-bp product yielded a 99% match with two sequences identified as *G. basitruncatum*. These included AB038427, derived from JCM 9300 (ex-type culture), and AB038425, derived from strain JCM 8083.

Antifungal susceptibility testing was performed by the CLSI-approved method (3). The MICs for the case isolate were as follows: amphotericin B, 0.5 μg/ml; itraconazole, >16 μg/ml; voriconazole, 8 μg/ml; caspofungin, 2 μg/ml; ketoconazole, 4 μg/ml; fluconazole, >64 μg/ml; flucytosine, >64 μg/ml.

Discussion. *Graphium basitruncatum* has not been reported previously as a human pathogen. We describe infection in an immunocompromised patient from whom this organism was repeatedly isolated from a sterile source (blood) and metastasized to the skin, resulting in necrotic fungal nodules. The patient was initially treated with caspofungin and voriconazole empirically. Recurrent fungemia while on this therapy is consistent with the high in vitro MICs (2 μg/ml and 8 μg/ml, respectively). The patient had clinical improvement with liposomal amphotericin B, although the improvement also coincided with recovery of neutrophils and was temporary. He then died from a relapse of his infection, despite showing no evidence of leukemia recurrence.

Fungemia in immunocompromised patients is primarily due to yeasts, such as *Candida* sp., *Cryptococcus* sp., or *Trichosporon* sp., and is much less common with molds. However, filamentous fungi such as *Aspergillus*, *Fusarium*, *Exophiala*, and *Phaeoacremonium* spp. may cause bloodstream infections and cutaneous lesions in this patient population. Fungemia due to *Graphium basitruncatum* has not been previously reported in the literature.

The biology and distribution of *Graphium basitruncatum* are poorly known. The fungus has been isolated twice from soil, including from the original location in the Solomon Islands, and from Japan (JCM 8083). Described originally as *Stilbum basitruncatum* by Matsushima (9), the species was later considered a synonym of *Graphium penicillioides* by Sutton (15). It is possible, therefore, that other collections are classified as *G. penicillioides*, a widely distributed species for which the substrate is predominantly wood. Sutton reported the latter to be common in wood and beetle tunnels in *Ulmus americana* (American elm) in two Canadian provinces (Manitoba and Saskatchewan). How our patient acquired his infection is unknown. He lived in a rural area of Ontario, Canada, where he regularly worked in his yard and pruned trees. There was no evidence of a recent wound by thorn or splinter, although he had sustained several cuts in the past. He had no recent travel history.

The genus *Graphium* includes synnematous fungi having annellidic and sometimes sympodial conidiogenesis and slimy conidia. Current taxonomic concepts restrict the genus to anamorphic members of the *Microascaceae* (11), a family of the *Ascomycota* that includes the important opportunistic pathogens *Pseudallescheria boydii* (*Scedosporium apiospermum*) and *Scedosporium prolificans* (4, 6, 12). *Graphium*-like species having affinities to the family *Ophiostomatales* are placed in the genus *Pesotum* (10). Okada et al. determined that *Graphium penicillioides*, the type species, was a species aggregate and that *Stilbum basitruncatum* was morphologically and phylogenetically distinct (10, 11). They proposed a new combination as *G. basitruncatum* (Mats.) Seifert and Okada and selected an epitype specimen for *G. penicillioides* (11). Teleomorphs for *Graphium* species occur in *Pseudallescheria* and *Petriella*, but no teleomorph for *G. basitruncatum* is known. In a phylogenetic analysis based on analysis of internal transcribed spacer sequences, *G. basitruncatum* (shown in the tree as "*G. penicillioides*" CBS 320.72) was basal to all *Pseudallescheria* and *Petriella* species and the anamorphic *S. prolificans* (4, 13). Analyses including more *Graphium* species are needed to evaluate the relationships among these fungi.

Graphium synnemata are uncommonly observed in the clinical laboratory; their development is enhanced by subculture on sporulation media such as oatmeal salts agar. *Graphium* states are usually found associated with *Scedosporium* synanamorphs in isolates of *P. boydii* and, less commonly, *Petriella* species. Conidial shape and tolerance to cycloheximide may help to distinguish these fungi from *G. basitruncatum*. Synnematal conidia of *P. boydii* and *Petriella* species are cylindrical to broadly clavate (mostly greater than 2.5 μm in width), with flattened bases. Those of *G. basitruncatum* are narrow (less than 2.2 μm in width) and slightly curved (allantoid), with narrow, truncate bases (shown in Fig. 2 and in scanning electron micrographs published in references 10 and 11). Brown oval conidia also may be produced but differ by having rounded bases. Isolates of *P. boydii* are usually tolerant to cycloheximide at a concentration of 0.4%; however, variability in tolerance has been reported (6). *P. boydii* is now recognized as a species aggregate, and it is unknown whether the observed variation correlates with molecular subgroups (12). *G. basitruncatum* and *S. prolificans*, which lacks both a *Graphium* state and a teleomorph, are completely inhibited at this concentration of cycloheximide. Although the name *G. eumorphum* has been used for the synnematal state of *P. boydii* (2), *Graphium* states are found only among some subgroups of the *P. boydii* aggregate; it is not yet clear to which subgroup this name applies (12).

It seems likely that *P. boydii* was the fungus discussed in two prior studies that attributed infections to *Graphium* species. A case of endophthalmitis involved a patient with underlying rheumatoid arthritis and chronic anterior uveitis that had been treated with topical and systemic corticosteroids (2). The fungus is described as having round and oblong conidia, characteristics suggestive of *P. boydii*. Similarly, disseminated infection in a dog was attributed to *G. fructicola*, but the published illustrations indicate that the fungus involved was *P. boydii* (8).

The optimal therapy for *Graphium basitruncatum* systemic infection is unknown. *Scedosporium* species and *G. basitruncatum* appear to demonstrate similar responses to antifungal drugs in vitro, except for amphotericin B, which showed good activity against the clinical isolate of the latter species (5). Clinical improvement in our patient's case was dependent on improvement in his immune status. The fatal outcome for this patient suggests that systemic invasive infections caused by this

fungus may have a high mortality rate, similar to that which occurs with *Scedosporium* infections.

Nucleotide sequence accession number. A sequence from the case isolate UAMH 10611 was deposited in the GenBank database under accession number EF165016.

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